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First record of *Macrobrachium mammillodactylus* (Thallwitz, 1891) (Crustacea, Decapoda, Palaemonidae) from Japan

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Abstract. Knobtooth Prawn, *Macrobrachium mammillodactylus* (Thallwitz, 1891), is an amphidromous freshwater prawn that occurs in the tropics of the western Pacific Ocean. Here, we report a new record of this species from Miyako Island, in the Ryukyu Archipelago, Japan. The obtained specimens were mature, suggesting that they had overwintered. This record updates the northern limit of *M. mammillodactylus* and suggests an expansion of the range of this species.

Keywords. Amphidromous, DNA barcoding, mitochondrial DNA, Ryukyu Archipelago

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Introduction

The freshwater shrimp genus *Macrobrachium* C.S. Bate, 1868 has more than 270 known species, with remarkably high species diversity in tropical freshwaters (De Grave and Fransen 2011; DecaNet Eds. 2023). Most of the widely distributed species in the Pacific have amphidromous migratory life histories and can expand their distributions by dispersing with ocean currents (Wowor et al. 2009). These highly dispersive species are likely to rapidly respond to the environmental changes caused by global climate change by shifting their distribution ranges (Pecl et al. 2017). However, there is limited information on the ranges of most freshwater species, which has been a limiting factor in studies on responses to environmental change (Ficke et al. 2007). Therefore, species' distribution records are becoming increasingly important. Here, we report *Macrobrachium mammillodactylus* (Thallwitz, 1891) from Miyako Island, Ryukyu Archipelago, which represents the first record from Japan. This is the northern known occurrence of *M. mammillodactylus*, suggesting an expansion of the range of this species.

Methods

Two specimens of *Macrobrachium mammillodactylus* were collected by hand net from the Sakita River, Miyako Island, Ryukyu Archipelago, Japan (Fig. 1) and preserved in 70% ethanol. The examined specimens have been deposited at the Kanagawa Prefectural Museum of Natural History, Japan (KPM).

The genus *Macrobrachium* shows marked sexual dimorphism, so most of the diagnostic characteristics are found only in males. The two specimens obtained in this study were female, so DNA barcoding based on mtDNA was first performed.

Total DNA was extracted from the fifth pleopod with the Monarch Genomic DNA Purification Kit (New England Biolabs, USA). A fragment of the mitochondrial 16S rRNA gene was amplified using PCR with the primer pairs 16S-F1 (5′-GTA CCT TTT GTA TCA GGG-3′) and 16S-R1 (5′-CGG TYT GAA CTC AAA TCA TG-3′) (this study). The composition of the 15 μL reaction mixture was as follows: 0.3 μL of PrimeSTAR GXL DNA Polymerase (TaKaRa, Japan), 3.0 μL of 5× PrimeSTAR GXL Buffer, 1.2 μL of dNTP Mixture,

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0.9 µL of each 5 µM primer, 1 µL of template DNA, and 7.7 µL of ultrapure water. The PCR amplification conditions were as follows: initial denaturation (98 °C, 30 s); 30 cycles of denaturation (98 °C, 10 s), annealing (55 °C, 15 s), and extension (68 °C, 45 s); and a single final extension (68 °C, 30 s). The PCR products were purified using illustra ExoProStar (Cytiva, USA) and outsourced to Eurofins Genomics, Japan, for Sanger sequencing.

The obtained sequences were checked for quality and manually edited using the mapping function of Unipro UGENE 47.0 (Okonechnikov et al. 2012). For DNA barcoding, similarity searches of nucleotide sequences were performed using BLASTN (Altschul et al. 1990; Johnson et al. 2008) with default settings. The sequences were deposited in DDBJ/EMBL/GenBank (accession numbers: LC777273–LC777274).

In addition, to assess the morphological characteristics of the specimens, the postorbital carapace length (CL) and segments of the first to third pereiopods were measured after preservation with digital calipers (CD67-S20PM; Mitutoyo, Japan). Photographs of the specimens are registered in the ffish.asia (https://ffish.asia/?page=sampling&pid=9910), a biodiversity database of freshwater organisms in Asia (Kano et al. 2013).

Results

Order Decapoda
Family Palaemonidae Rafinesque, 1815
Genus *Macrobrachium* C.S. Bate, 1868

***Macrobrachium mammillodactylus* (Thallwitz, 1891)**
Figure 2

New Japanese name. イボユビテナガエビ [Ibo-yubi-tenagaebi]

Materials examined. JAPAN – **Okinawa** • Ryukyu Archipelago, Miyako Island, Sakita River; 24°45′11.6″N, 125°17′10.1″E; 01.X.2016; Tomoaki Maruyama leg.; GenBank: LC777273, LC777274; 2♀, CL 23.6 mm and 18.4 mm, KPM-NH 2940, 2942.

Identification. Although the diagnostic characteristics of the males were unavailable, the two female specimens examined were identified as *M. mammillodactylus* based on genetic evidence. A similarity search of 869 bp of 16S rRNA from our specimens using BLAST showed 98.6–100% identity with the sequences from specimens identified as *M. mammillodactylus* in previous studies (Table 1). The sequences of *Macrobrachium idae* (Heller, 1862), a species morphologically similar to

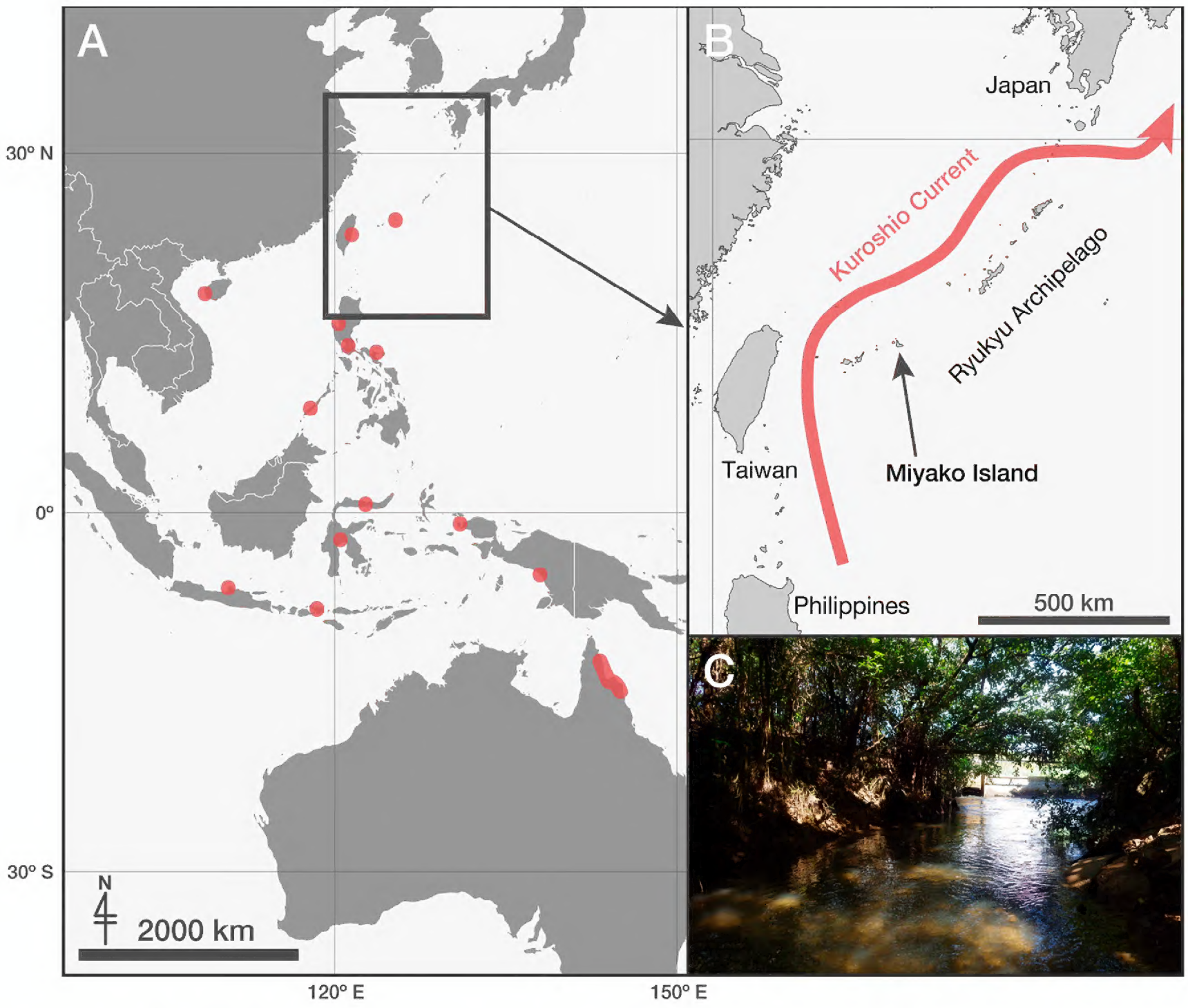

Figure 1. Range and collection site of *Macrobrachium mammillodactylus*. **A.** Distribution map. Red points represent the occurrence of the species based on the literature record. **B.** Enlarged map around the Ryukyu Archipelago. **C.** Habitat of *M. mammillodactylus* on Miyako Island.

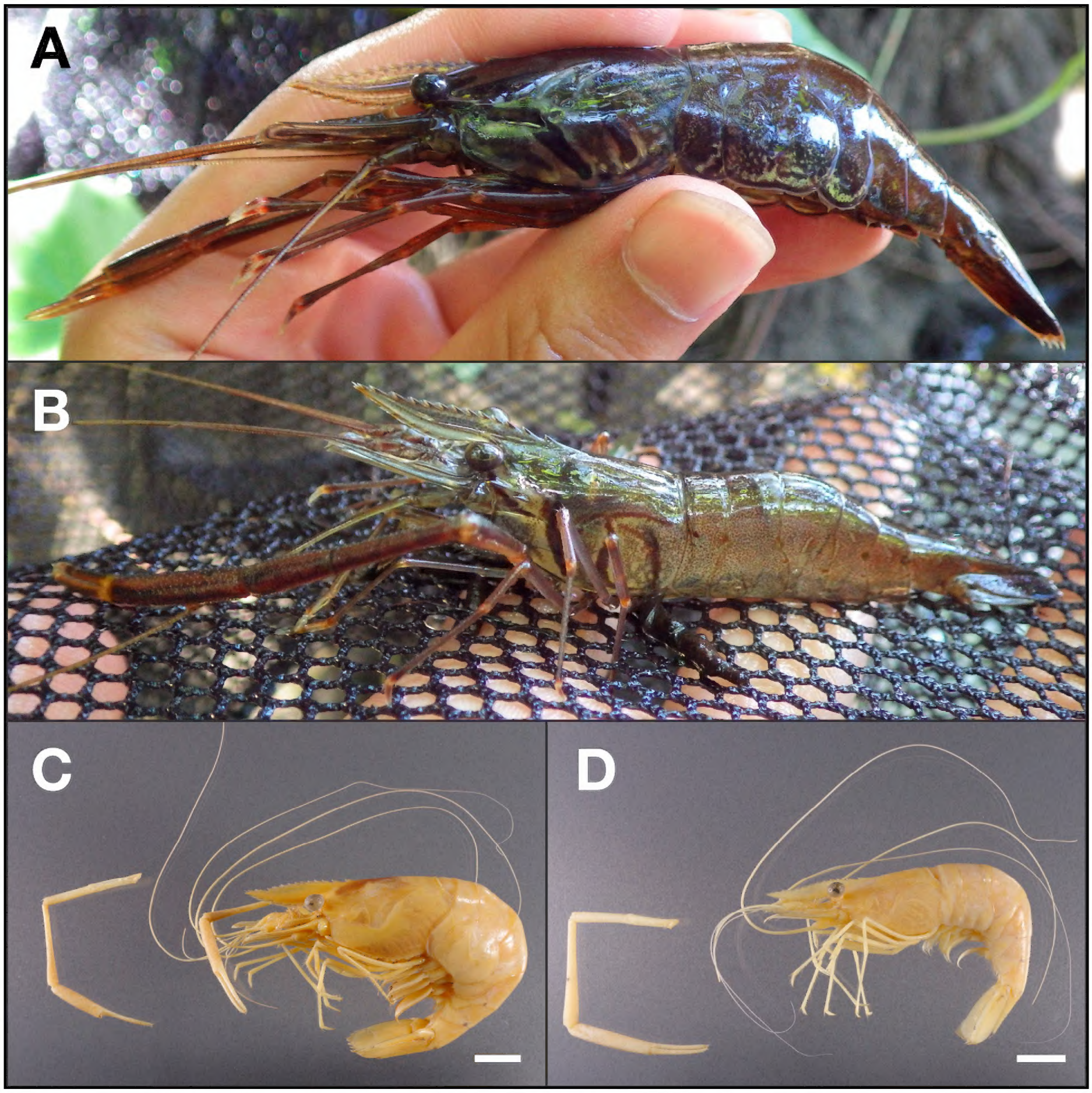

Figure 2. *Macrobrachium mammillodactylus* collected from Miyako Island, Japan. **A.** Live condition of KPM-NH 2940. **B.** Live condition of KPM-NH 2942. **C.** Lateral view of KPM-NH 2940 after fixing. **D.** Lateral view of KPM-NH 2942. Scale bars: C, D = 10 mm.

M. mammillodactylus, matched 94.0–94.5% and were genetically distinct from *M. mammillodactylus*.

The morphological characteristics of the specimens were consistent with those of *M. mammillodactylus*: the rostrum is straight and with a formula of 3+10/4–5 (Chace and Bruce 1993), and the chela of the second pereiopods lacks setal pubescence and is longer than the carpus (Short 2004). The distal two and posterior two rostral teeth on the dorsal margin were slightly spaced from the other teeth, which is consistent with the findings of previous studies (Cowles 1914; Short 2004). This feature does not appear in old males (Cowles 1914) but was confirmed in adult females.

Morphology. The specimens collected from Japan were female. Rostrum straight and its tip slightly upward, reaching distal end of scaphocerite; dorsal margin with 10 teeth, including three on carapace posterior to orbital margin, anterior two and posterior-most two teeth slightly further apart than the others; ventral margin with 4 or 5 teeth. First pereiopod slender; finger 0.87–0.96 times shorter than manus (palm); chela 0.37–0.47 times shorter than carpus; carpus 1.20–1.38 times longer than merus; merus 1.92–2.08 times longer than ischium. Second pereiopod slender, equal in shape and size, 2.8–4.2 times longer than CL; tips of finger curved inwards with no setal pubescence, finger 0.68–0.90 times shorter than palm; chela 1.26–1.34 times longer than carpus; carpus 1.52–1.72 times longer than merus; merus same length as ischium. Third pereiopod slender; dactylus 0.32–0.34 times shorter than propodus; propodus 1.96–2.00 times longer than carpus; carpus 0.47–0.49 times shorter than merus; merus 2.25–2.83 times longer than ischium.

Color. Body and pereiopods brown to translucent, with

Table 1. BLAST search results for *Macrobrachium mammillodactylus*. Shows the query with >95% identity.

Query sample	Hit species	Length (bp)	Query cover	Identity	Accession	References
KPM-NH 2940 (LC777273)	*M. mammillodactylus*	533	56%	99.80%	EU493148	Chen et al. 2009
		519	56%	99.80%	FM986629	Wowor et al. 2009
		446	50%	99.77%	AY377845	Murphy and Austin 2005
		528	55%	99.58%	DQ194915	Liu et al. 2007
		446	50%	99.32%	AY282776	Murphy and Austin 2005
		442	49%	98.84%	JF310724	Page and Hughes 2011
		442	49%	98.60%	JF310723	Page and Hughes 2011
KPM-NH 2942 (LC777274)	*M. mammillodactylus*	533	56%	100.00%	EU493148	Chen et al. 2009
		519	56%	100.00%	FM986629	Wowor et al. 2009
		446	50%	100.00%	AY377845	Murphy and Austin 2005
		528	55%	99.79%	DQ194915	Liu et al. 2007
		446	50%	99.55%	AY282776	Murphy and Austin 2005
		442	49%	99.07%	JF310724	Page and Hughes 2011
		442	49%	98.84%	JF310723	Page and Hughes 2011

three distinct lateral lines on carapace and a gentle narrow wavy line extending from postorbital angle to the longitudinal posterior of carapace (Fig. 2A, B). Joints of pereiopods orange except between carpus and chela of second pereiopod.

Habitat. *Macrobrachium mammillodactylus* specimens were collected from tree roots submerged in freshwater in the lower reaches of the river (Fig. 1C). The water temperature at the time of collection was 26.5 °C. *Macrobrachium formosense* C.S. Bate, 1868, *Caridina serratirostris* De Man, 1892, and *Caridina typus* H. Milne Edwards, 1837 were collected sympatrically.

Distribution. Japan (present study), Taiwan (Chen et al. 2009a, 2009b), China (Liu et al. 1990), Philippines (Holthuis 1950; Chace and Bruce 1993; Cai and Shokita 2006; Cuvin-Aralar 2014), Indonesia (Holthuis 1950; Chace and Bruce 1993; Jurniati 2021), and Australia (Short 2004).

Etymology of Japanese name. The new Japanese name for *M. mammillodactylus* means "warty finger" and is derived from the rows of tubercles on the cutting edge of the chela of adult males (Thallwitz 1891; Roux 1923). Specimen KPM-NH 2940 was designated as the standard specimen for the new Japanese name for *M. mammillodactylus*.

Discussion

This study provides the northernmost record of the distribution of *Macrobrachium mammillodactylus* and suggests a northward expansion of this species via the Kuroshio Current. This species has been previously reported in Taiwan, the Philippines, Indonesia, and Australia (Holthuis 1950; Chace and Bruce 1993; Short 2004; Chen et al. 2009). Dispersal of this species to the Ryukyu Archipelago would have been possible because it has a marine larval phase (Short 2004; Wowor et al. 2009). However, the Ryukyu Archipelago has historically been an area of active research on freshwater prawns, but there have been no records of this species (e.g. Kubo 1940; Shokita 1975, 1979; Yoshigou 2002). Although identification of individuals other than adult males based on morphology is difficult for this species, and juveniles and females may have been missed in previous surveys, the occurrence of adults and the suggestion of overwintering of this species is clearly a first.

Since the Sakita River where this species was found is spring origin, water temperatures may be stable throughout the year, although no data are available. Such an environment is also present on other major islands of the Ryukyu Archipelago, and future surveys may find this species on other islands. Clarification of the morphological characteristics of juveniles is needed to accurately identify this species in the field. As an alternative approach, species detection using environmental DNA with metabarcoding or species-specific primers (Komai et al. 2019) would be useful to understand the range of this species.

The northern range expansion of this species has also been reported for other amphidromous freshwater prawns, for example *Macrobrachium australe* (Guérin-Méneville, 1838) in mainland Japan (Imai et al. 2008), *Macrobrachium latimanus* (von Martens, 1868) in mainland Japan (Maruyama 2018), *Macrobrachium placidulum* (De Man, 1892) on Kuchinoerabu Island, Ryukyu Archipelago (Sanuki et al. 2019), and *Macrobrachium ustulatum* (Nobili, 1899) on Okinawa Island, Ryukyu Archipelago (Fuke and Imai 2018). Global temperature increases, that is +1.51 °C/100 years on Miyako Island (Okinawa Meteorological Observatory 2022), may explain the expanding distribution of these species. Accumulation of such fundamental data on the distribution of amphidromous species will provide insights into range shifts in the future.

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Author Contributions

Conceptualization: YF, TM. Data curation: YF. Formal analysis: YF. Funding acquisition: YF. Investigation: TM. Visualization: YF. Writing – original draft: YF. Writing – review and editing: YF, TM.

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